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Are lab-cultured *Anaphes iole* females strictly proovigenic?

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Abstract. *Anaphes iole* Girault (Hymenoptera: Mymaridae) is a solitary egg parasitoid of *Lygus* bugs (Heteroptera: Miridae) in North America. Circumstantial evidence suggests that *A. iole* females are strictly proovigenic. This study was designed to determine if honey-fed *A. iole* females could in fact mature additional eggs if exposed to hosts for a few days then removed from hosts and held at 25 °C for 0, 3, or 6 days. Contrary to expectation, honey-fed *A. iole* females matured considerably more eggs when deprived of hosts for 3 or 6 days rather than 0 days. This research suggests that *A. iole* females are not strictly proovigenic. However, they do have proovigenic tendencies, since most females emerge with at least 71% of their potential fecundity.

Key words: *Anaphes iole*, egg load, egg parasitoid, *Lygus hesperus*, oviposition behavior

Introduction

Anaphes iole Girault (Hymenoptera: Mymaridae) is an important egg parasitoid of *Lygus* bugs (Heteroptera: Miridae) in the United States (Jones and Jackson, 1990; Jackson, 2003) and has potential for reducing populations of *Lygus hesperus* Knight on small fruits in California (Udayagiri et al., 2000). This parasitoid may also be useful in suppressing *Lygus shulli* Knight and *Lygus elisus* Van Duzee populations on vegetables (e.g., cucumbers, sweet peppers) in greenhouses in southwestern British Columbia (McGregor et al., 2000).

A prerequisite to inundative releases of *A. iole* is the development of an efficient mass rearing system. Previous research has suggested that exposing newly emerged, mated *A. iole* females to a moderate to high density of *L. hesperus* eggs for a few days could lead to time-efficient production of adult progeny in an *in vivo* rearing system (Riddick, 2003, 2004). Knowledge of the egg load (i.e., quantity of mature eggs contained within the ovaries) of newly emerged *A. iole* might be useful for estimating production potential of this parasitoid.

Riddick (2005) indicated that the egg load of newly emerged *A. iole* was approximately 48 mature eggs before exposure to hosts and that 86–92% of females contained less than 6 mature eggs and no immature eggs after 1, 3 or 5 days of exposure to hosts. Also, egg load was not related to body size or affected significantly by age of *A. iole* females.

The relationship between time of egg maturation and parasitoid oviposition on hosts can be used to group females into two categories: (1) proovigenic species, in which egg maturation is complete or nearly complete before females begin ovipositing; and (2) synovigenic species, in which egg maturation is not complete at the onset of oviposition, but is more or less continuous throughout the life of the female (Flanders, 1950). In reality, most species fit along a continuum between the extremes of strict proovigeny and strict synovigeny (Jervis and Copland, 1996). Presumably, a strictly proovigenic species emerges with a full complement of mature eggs and is incapable of maturing additional eggs, even after oviposition ensues. In contrast, a strictly synovigenic species emerges with few or no mature eggs and must mature its eggs throughout its lifetime. Jervis et al. (2001) established an 'ovigeny' index (i.e., the fraction of the maximum lifetime complement of mature eggs that a parasitoid female possesses upon emergence), based on a scale of 1 for strict proovigenic species to 0 for strict synovigenic species. Purportedly, mymarid wasps such as *A. iole* (= *A. ovijentatus* (Crosby and Leonard)), *Anagrus* sp., *Anagrus sophiae* S. Trjapitzin (= *A. delicatus* Dozier, Trjapitzin and Strong, 1995), *Anagrus giraulti* Crawford and *Anagrus erythroneurae* Trjapitzin & Chiappini, have an ovigeny index of 1 (Jervis et al., 2001). Note that strict proovigeny is rare or non-existent in parasitoid wasps in other families (Ellers and Jervis, 2003). Proovigenic species are usually short-lived and feed little (Flanders, 1950); it is advantageous for proovigenic females to lay the majority of their eggs within several days after emergence (Heimpel and Rosenheim, 1998).

Research has indicated that purportedly proovigenic species can mature additional eggs after emergence from hosts. For example, Chantarasa-ard et al. (1984) stated that egg production continued during the adult lifespan of some *Anagrus incarnatus* Haliday females, when fed honey and reared at 20 °C. Santolamazza Carbone and Cordero Rivera (2003) found several more mature eggs in *Anaphes nitens* Girault females that were deprived of hosts and fed honey for 2 days than in conspecific females that were removed from hosts and killed directly. They concluded that *A. nitens* females were weakly synovigenic.

Whether or not *A. iole* females are capable of maturing additional eggs after being deprived of hosts for several days is unknown.

I designed an experiment to test if honey-fed *A. iole* females could in fact mature additional eggs when deprived of hosts for 0, 3, or 6 days. The results should clearly determine if *A. iole* is strictly proovigenic or not.

Materials and methods

Insect cultures

Lygus hesperus was initially obtained from Biotactics, Inc. (Riverside, CA) and was reared continuously for approximately 7 years at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS. Adults and nymphs were reared on artificial diet (Cohen, 2000). *Anaphes iole* was initially obtained from Biotactics, Inc. (Riverside, CA). Adults were reared continuously from *L. hesperus* eggs for approximately 4 years at the USDA-ARS, BCMRRU, Mississippi State, MS. Details of the rearing protocols for both species have been published previously (see Riddick, 2003).

Effect of host deprivation on egg load

This experiment was designed to determine if the time (0, 3, or 6 days) that parasitoids were deprived of hosts could affect the egg load of *A. iole* females. A randomized block design was used and each female was considered an independent sampling unit. There were 4 replicate females for each time treatment group, for a total of 12 females per trial. There was a single host patch (containing in excess of 100 *L. hesperus* eggs) for each treatment group for a total of 12 patches per trial. Trial date (18 May and 3 June 2004) was used as a blocking factor.

Parasitoid age was standardized and mating was facilitated by placing late stage *A. iole* pupae inside a gelatin capsule ($\sim$7.5 mm $\times$ 20 mm); two per capsule. The sex of occupants in each capsule was determined on the same day that adults emerged from hosts. Females with mates were not observed long enough to ensure that mating had occurred within the capsules. But, 100% of 0- and 1-day-old *A. iole* females generated female progeny when confined, individually, with a male of similar age and with hosts for 24 h in a previous study (Riddick, 2003). Unmated *A. iole* females produce only male progeny.

Lygus hesperus eggs, partially embedded in patches (9 cm $\times$ 15 cm), were obtained from the colony at BCMRRU (see *Insect cultures*). Eggs had been oviposited in patches within 3 h of the onset of each trial. Each host patch was placed at the base of a plastic Petri dish

(15 mm × 100 mm) with lid. Approximately 2 μl of honey (20%, v/v aqueous solution) was applied to the four corners of each host patch; and approximately 4 μl of honey (20%) was streaked on the lid of each Petri dish to provide a food source to extend the lifespan of ovipositing females. Honey-fed *A. iole* females do not oviposit significantly more eggs than unfed females within 48 h (Riddick, 2003, 2004). Next, one (0-day-old) *A. iole* female without its mate (0-day-old male cohort) was carefully released into the Petri dish containing a host patch and placed in a growth chamber (25.0 °C, 60–70% RH, and 16 h photophase, using 20-Watt fluorescent lights).

Time of exposure to hosts was limited to 48 h for all parasitoids; then each host patch was removed and placed inside a clean Petri dish. Female parasitoids remained inside the original dish inside the same growth chamber for 0, 3, or 6 days. After completion of the designated time of host deprivation, adult females were removed from the growth chamber and stored (inside the original Petri dish) in a counter-top freezer (−18 to −20 °C) until dissection. All female parasitoids were still alive after the end of designated treatment time periods prior to placement in the freezer.

For dissection, wasps were placed on a glass microscope slide in a drop of saline solution (i.e., 6.5 g NaCl/l) and observed under a stereo-zoom dissecting microscope (Olympus, model #SZ11) at a magnification of 60–90X. Using two #0 insect micropins, the abdomen of each female was teased apart and ovaries removed. Next, a glass coverslip was gently placed over the ovaries. Applying slight pressure on the coverslip caused the individual eggs to become discernible. This procedure was necessary because the eggs were too tightly packed within the ovaries for accurate counting of the eggs. This procedure did not rupture the chorion of the eggs (i.e., the eggs remained intact). A digital imaging software program, Image-Pro Plus (1999) and an inverted system microscope (Olympus, model #IX70) was used for counting all ovarian eggs at a magnification of 100–400X. Estimates of body size of females were not taken in this experiment, since a recent study (Riddick, 2005) indicated that neither hind tibia nor forewing length affected the initial egg load of *A. iole* females. A total of 24 females were dissected in this experiment. The number of ovarian eggs remaining in *A. iole* females were determined for each treatment group. Only mature eggs (i.e., fully chorionated oöcytes) were detected. The size of *A. iole* mature eggs was not recorded in this study. However, all were of the same approximate size and consisted of a body and pedicel as typical of mature eggs of other mymarid species (see Jackson, 1969; King and Copland, 1969; Moratorio and Chiappini, 1995; Jervis and Copland,

1996). The number of mature eggs per female was correlated with the number of progeny (late stage pupae) produced by the same female when on its host patch.

Statistical analysis

A general linear model (GLM) with analysis of variance (ANOVA) was used to test the significance of time of host deprivation and trial date on the number of mature eggs present inside *A. iole* females. Pearson's Product–Moment correlation was used to determine if there was a significant association between the number of mature eggs per female and the number of progeny produced by the same female, before being removed from host patches. ANOVA was used to determine if the number of progeny produced by parasitoids and the number of hosts available per patch differed significantly between treatment groups. Data were square-root transformed prior to analysis (Zar, 1999). Mean values were considered significantly different when $p < 0.05$. Tukey's method was used for pairwise comparisons of means. Data analyses were performed with SigmaStat (2004) or SYSTAT (1998) computer software. Only untransformed data are presented.

Results and discussion

The time that *A. iole* females were deprived of hosts had an impact on subsequent egg load (Figure 1a); significantly more mature eggs were present in parasitoids after 3 and 6 days than after 0 days ($F = 10.66$; df = 220; $p = 0.0007$). Trial date had no effect on egg load ($F = 0.76$; df = 1, 20; $p = 0.39$). Mature egg load ranged from 0–12 eggs (median, 1.0), 0–30 eggs (median, 20.5) and 14–26 eggs (median, 19.0) for those parasitoids isolated from host patches for 0, 3 or 6 days, respectively. Immature eggs were not detected in any of the females.

There was no correlation between the egg load of *A. iole* females and the number of progeny (late stage pupae) that these same females had produced, within a span of 48 h on host patches (0 day group: $r = -0.31$; $p = 0.45$; n = 8; 3 days group: $r = -0.06$; $p = 0.89$; n = 24; 6 days group: $r = -0.21$; $p = 0.62$; n = 8; Figure 1b). The mean ± SEM number *A. iole* progeny produced from each host patch was 39.0 ± 6.2, 42.25 ± 2.3 and 44.75 ± 3.1 for 0, 3 and 6 days groups, respectively, and did not differ significantly between treatment groups ($F = 0.47$; df = 2, 21; $p = 0.63$). The mean ± SEM number of host eggs available per patch was 147.25 ± 12.0, 141.4 ± 5.7 and 137.9 ± 9.1 for 0, 3 and

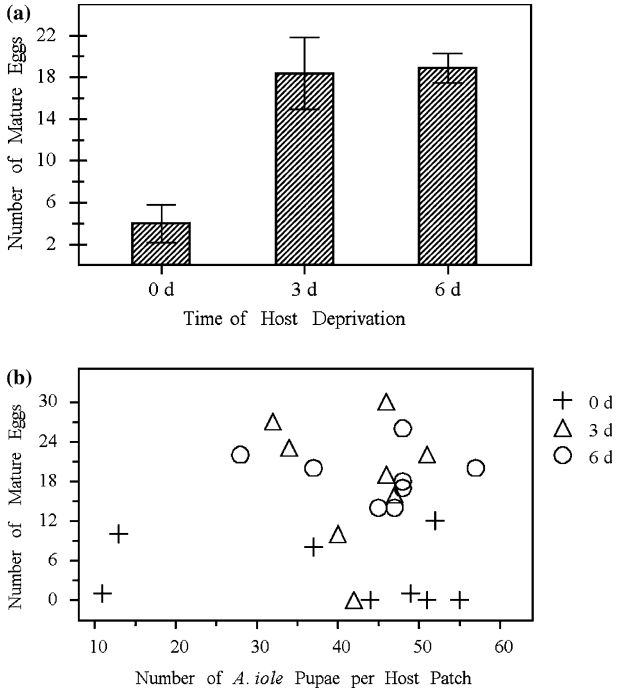

Figure 1. Mean ± SEM number of mature eggs found in the ovaries of *A. iole* females at 0, 3, or 6 days of host (*L. hesperus*) deprivation (a), and scatter plot of the correlation between the number of mature eggs and *A. iole* late stage pupae per patch (b) n = 24 *A. iole* females, eight per treatment group, for detection of mature eggs. For the correlation analysis, n = 24 host patches.

6 days groups, respectively, and did not differ significantly between treatment groups ($F = 0.26$; df = 2, 21; $p = 0.78$).

The observation that progeny production was not correlated with subsequent egg load and that *A. iole* females contained an average of 18 or 19 mature eggs after 3 or 6 days away from host patches, suggests that this parasitoid is capable of maturing additional eggs, regardless of the number of eggs deposited earlier on host patches. But, further egg development and maturation might be dependent upon two conditions: (1) *A. iole* females must be fed to prolong their lifespan (see Riddick, 2003) before oogenesis can be maximized, and (2) females have to dump some of their initial egg load in order to free-up space in the ovaries for development and then storage of new eggs (see Mills and Kuhlmann, 2000). The process of removing females from their hosts may have provided time for new eggs to mature and accumulate in the ovaries. This removal method was used by Santolamazza Carbone and Cordero

Rivera (2003) to demonstrate that *A. nitens* females were capable of maturing additional eggs; even though immature eggs were not detected.

The previous assignment of an ovigeny index of one to *A. iole* (see Jervis et al., 2001) should be re-evaluated. Although lab-cultured *A. iole* females are not strictly proovigenic, as demonstrated in this study, they can be considered as mostly proovigenic, because they are closer to the proovigenic end of the continuum (between extremes of proovigeny and synovigeny) than the synovigenic end. If *A. iole* females contain 44 mature eggs upon emergence and then have the capability of maturing 18 additional eggs, after exposure to and then removal from hosts, 62 eggs would represent the potential lifetime fecundity for lab-cultured *A. iole*, assuming that no further egg maturation occurs after 6 days. In this example, *A. iole* females would emerge with approximately 71% of their potential egg load available for oviposition into hosts. Thus, lab-cultured *A. iole* females would have an ovigeny index of 0.71.

Riddick (2003) stated that in an *in vivo* rearing system, time-efficient production of progeny may result when newly emerged, unfed *A. iole* females are exposed to suitable hosts for just a few days, since mated and virgin females deplete most of their egg load within 24 h. This current study suggests that it might be possible to recycle the females so as to nearly or fully exploit their potential fecundity. One rearing scenario could involve exposing unfed *A. iole* females to *L. hesperus* host patches for 24 h, then removing all host patches and provisioning cages with a food source (e.g., honey). The same *A. iole* females would remain in the honey-provisioned cages for 3 days; afterwards, freshly laid host patches could be presented to these females to parasitize in 24 h. Thus, the same cohort of females would be given the opportunity to parasitize hosts on two separate occasions. This scenario appears reasonable, but its utilization will depend on (1) whether it is more time and cost-efficient to recycle females over several days rather than throw them away after 1 day to provide space for newly emerged females, and (2) whether the new eggs are viable and give rise to adults of unbiased or female-biased sex ratios.

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